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Brain network interactions in transgender individuals with gender incongruence

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ABSTRACT

Functional brain organization in transgender persons remains unclear. Our aims were to investigate global and regional connectivity differences within functional networks in transwomen and transmen with early-in-life onset gender incongruence; and to test the consistency of two available hypotheses that attempted to explain gender variants: (i) a neurodevelopmental cortical hypothesis that suggests the existence of different brain phenotypes based on structural MRI data and genes polymorphisms of sex hormone receptors; (ii) a functional-based hypothesis in relation to regions involved in the own body perception. T2*-weighted images in a 3-T MRI were obtained from 29 transmen and 17 transwomen as well as 22 cisgender women and 19 cisgender men. Resting-state independent component analysis, seed-to-seed functional network and graph theory analyses were performed. Transmen, transwomen, and cisgender women had decreased connectivity compared with cisgender men in superior parietal regions, as part of the salience (SN) and the executive control (ECN) networks. Transmen also had weaker connectivity compared with cisgender men between intra-SN regions and weaker inter-network connectivity between regions of the SN, the default mode network (DMN), the ECN and the sensorimotor network. Transwomen had lower small-worldness, modularity and clustering coefficient than cisgender men. There were no differences among transmen, transwomen, and ciswomen. Together these results underline the importance of the SN interacting with DMN, ECN, and sensorimotor networks in transmen, involving regions of the entire brain with a frontal predominance. Reduced global connectivity graph-theoretical measures were a characteristic of transwomen. It is proposed that the interaction between networks is a keystone in building a gendered self. Finally, our findings suggest that both proposed hypotheses are complementary in explaining brain differences between gender variants.

1. Introduction

Gender identities are classified into "transgender" and "cisgender" umbrellas (Polderman et al., 2018). Transgender is an adjective used to describe a diverse group of individuals whose gender identity is different (in varying degrees) from the sex assigned to them at birth. Cisgender is used to refer to a gender identity that matches a person's sex assigned at birth. Nowadays, when an individual applies for transgender health care, they may receive a diagnosis based on fulfillment of DSM-5 (gender dysphoria, American Psychiatric Association, 2013) or ICD-11 criteria (gender incongruence, Reed et al., 2016). Both terms define a condition in which individuals experience a marked discrepancy between the sex

Abbreviations: CM, cismen; CW, ciswomen; DMN, default mode network; ECN, executive central network; ICA, independent component analysis; ROI, region of interest; SN, salience network; TM, transmen; TW, transwomen.

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assigned at birth and the gender they identify with.[1] Brain functional organization differences between cis men (CM) and cis women (CW) are extensively described in the literature, but studies on the expression of these differences in gender incongruence are scarce (Guillamon et al., 2016; Kreukels and Guillamon, 2016). However, the comparative study of cis and transgender persons could help us to understand the functional brain networks in gender and its variants. Brain imaging studies on transgender populations are usually focused on individuals in which the intensity of gender discrepancy is extreme, their experienced gender is with the opposite sex, and apply or have received gender confirming treatments. Many of these people make a social transition from male-to-female (trans woman, TW) or from female-to-male (trans men, TM), through cross sex hormonal treatment and subsequent physical adaptation through surgery (Hembree et al., 2009; Selvaggi and Bellringer, 2011).

A first predominant hypothesis on the implication of hormones in brain sexual differentiation and the development of gender was pointed out by Dr. Swaab (Swaab, 2004; Zhou et al., 1995). More recent studies performed in our group with brain MRI and genetic polymorphisms on hormone receptors were in line of this hypothesis and refined it. Using structural MRI techniques, and controlling for the pre-pubertal onset of gender incongruence and sexual attraction of hormonally-naïve transgender people, we studied white matter microstructure (Rametti et al., 2011a, 2011b) and cortical thickness (Zubiaurre-Elorza et al., 2014, 2013) differences of TM and TW in comparison with cisgender groups. Whereas TM had a morphological brain mix of masculine, feminine, and defeminized traits, TW showed a combination of feminine, masculine, and demasculinized traits. Moreover, TM, TW and CW presented thicker cortex than CM (Zubiaurre-Elorza et al., 2014). These findings prompted us to suggest a neurodevelopmental cortical hypothesis to explain gender (Guillamon et al., 2016). Each variant of gender has a brain phenotype that might be due to differences in cortical development probably due to differences in the efficiency of sex hormone receptors (Guillamon et al., 2016; Raznahan et al., 2010). Recently, in a large population sample, we have shown that the genes polymorphisms of sex hormone receptors are associated to gender incongruence. Briefly, TW condition requires an androgen receptor gene polymorphism, which must be accompanied by a β-estrogen gene polymorphism while α and β gene polymorphisms are associated with TM with no interaction. Further, the β-estrogen receptor plays a key function in brain differentiation in humans (Fernández et al., 2018).

While we suggested a neurodevelopmental cortical hypothesis based on structural MRI findings on cortical thickness (Guillamon et al., 2016), another recent hypothesis based on functional MRI connectivity data proposes that gender incongruence could rest on a disconnection of fronto-parietal networks implicated in own-body self-referential processing, presenting thickening in the mesial prefrontal and precuneus cortex as the neuroanatomical correlates (Burke et al., 2017; Feusner et al., 2017; Manzouri et al., 2017; Manzouri and Savic, 2018). Gender incongruence may be unrelated to brain sex differences based on findings in networks involved in one's body perception (Feusner et al., 2017). However, sex differences between CM and CW in functional connectivity have been reported in large samples (Biswal et al., 2010; Gong et al., 2011; Ritchie et al., 2018; Tomasi and Volkow, 2012), also using machine learning classificatory algorithms (Zhang et al., 2018).

Interestingly, we had described structural sex differences in the same temporal, frontal, and parietal regions and their related fasciculi, to that related to the one's body perception hypothesis (Guillamon et al., 2016). This may indeed provide support for the hypothesis of the Karolinska group on gender incongruence (Feusner et al., 2017). Further supporting this structural underpinnings, we had also reported demasculinization of white matter microstructure in right and left superior longitudinal fasciculi, the anterior region of the cingulum, and fornix minor in TW, while these fasciculi are masculinized in TM (Rametti et al., 2011a, 2011b).

To date, several methodological approaches have been used to characterize the functional connectivity in transgender persons. Independent Component Analysis (ICA) is a common method to study the whole-brain functional networks from a data-driven approach. In the literature, there are some discrepancies when investigating brain network connectivity differences for both TM and TW. Decreased connectivity in anterior and posterior parts of the default mode network (DMN) has been found in untreated TM (Feusner et al., 2017; Manzouri and Savic, 2018) and TW (Manzouri and Savic, 2018) when compared with cisgender groups. On the other hand, two studies did not find these differences in the DMN (Clemens et al., 2017; Nota et al., 2017), in the salience network (SN) or in the executive control network (ECN) (Nota et al., 2017). Clemens et al. (2017) reported that TW had higher connectivity in the thalamus as a part of the right fronto-parietal network (i.e., ECN), when compared with ciswomen; while connectivity was lower in TW when compared to that in CM in the dorsolateral prefrontal cortex of the left frontoparietal network.

Seed-based approaches allow to examine connectivity strengths between two or more regions of interest. In an early single-case study, one untreated TM participant had similar functional connectivity to CW (Santarnecchi et al., 2012). More recently, using a similar technique CM showed stronger connectivity than CW and TM from the left amygdala to the motor cortex and the right amygdala to the hypothalamus whereas no differences were found between CW and TM. Moreover, TM showed weaker connectivity than CM and CW from their amygdala to the posterior cingulate, the fusiform cortex and the extriatal body (Manzouri et al., 2017). TM also had stronger connectivity in the right temporo-parietal seed in connection with the bilateral insula when compared to a pooled group of cisgender individuals (Burke et al., 2017). To our knowledge, no study has taken advantage of such technique to investigate the interaction between different brain networks instead of studying them individually or testing connectivity differences between *a priori* regions of interest (ROI).

Another way of characterizing functional networks has become possible through recent advances in mathematics that allow to study the integrity properties of large-scale networks such as graph theoretical models. Such models provide another view on the brain global function and thus complement seed-based approaches or localized functional connectivity such as low frequency fluctuations or regional homogeneity (Mueller et al., 2016). To date, two studies have described connectomic differences using graph theory analysis employing structural (Hahn et al., 2015) and functional (Lin et al., 2014) data. Hahn et al. (2015) reported no global connectivity differences, decreased intrahemispheric lobar connectivity ratios of subcortical-limbic areas for TM, and increased interhemispheric lobar connectivity for TW. Likewise, Lin et al. (2014) also found regional differences without concurrent global changes. However, their study pooled both groups of transgender participants and both cisgender groups thus not allowing for gender-specific findings.

These prior lines of research demonstrate that reconciling findings across studies, and importantly across methods, is presently hindered by lack of direct comparative data stemming from the same dataset. Therefore, in the present study we aimed to investigate: (1) the functional connectivity differences employing a whole-brain data-driven approach; (2) the intra and inter connectivity strengths between functional networks; and (3) the global connectivity differences with a graph-theoretical approach in homogeneous groups of TM, TW and their comparison CM and CW groups. Based on prior available data reviewed above, we had 3 main hypotheses. First, from our previous structural works (Guillamon et al., 2016) founded in a neurodevelopmental hypothesis, CM would present different strength in connectivity than CW, TM and TW groups; and either no statistical significant differences would be present between these three groups or either such differences would

[1] Because gender incongruence is no longer classified as a mental disorder, and as in Baldinger-Melich et al. (2019), we chose this term to characterize our transgender sample in the present study.

be subtler. Second, from the one's body perception hypothesis (Feusner et al., 2017; Manzouri et al., 2017), differences in connectivity strength would be found in regions related to the body perception process. Third, in addition to gender differences between transgender and cisgender groups, we would also find functional connectivity sex differences both globally and regionally in different brain networks such as the DMN, the SN, the ECN or the sensorimotor network (Gong et al., 2009; Ritchie et al., 2018).

Therefore, the novelty of the present study is based on an initial data-driven approach and in investigating the networks interrelations instead of examining them individually. Moreover, we tested the consistency of two hypotheses, the cortical developmental hypothesis (Guillamon et al., 2016) and the functional-based hypothesis in relation to regions involved in the own body perception (Feusner et al., 2017; Manzouri et al., 2017).

2. Methods

2.1. Participants

Thirty-four untreated TM and 21 untreated TW were enrolled and diagnosed at the Gender Identity Unit of the Hospital Clinic of Barcelona from September 2014 to November 2017. Fifty-six cisgender volunteers were recruited by advertising among the students enrolled in the first year of the Bachelor of Nursing program at the University of Barcelona, Campus Clinic. These students were also invited to recruit friends or relatives of similar age and education. The inclusion criteria for the transgender groups were: (1) presenting with gender incongruence according to the ICD-11 with an identification with the other gender (male or female); (2) presenting early-life onset of gender nonconformity before or at puberty; (3) being erotically attracted to persons with the same anatomical sex; (4) having no prior history of hormonal treatment before their MRI scan and (5) having no disorder of sexual development. For CM and CW, the inclusion criteria were that (1) individuals would be between 18 and 35 years old; and (2) identification as CM or CW. The exclusion criteria for all groups were: (1) presence of neurological or psychiatric disorders; (2) presence of hormonal disorders; (3) MRI incompatibilities such as having implanted metal objects that could not be extracted; (4) fulfilling DSM-5 criteria for substance use disorder within the past year and (5) current use of psychoactive medication.

At the time of recruitment, transgender people met diagnostic criteria for gender identity disorder (according to DSM-IV-TR and ICD-10) as assessed by means of several interviews by the experienced psychiatrist of the team (EGG) and psychologists as previously described (Gómez-Gil et al., 2009). To avoid stigmatization of mental disorders, diagnosis was relabeled to gender incongruence. In the present sample, changes do not interfere with the interpretation of our results, since transgender participants explicitly reported feelings of belonging to the opposite gender to the one assigned and expressed the desire for hormonal and surgical gender affirmation treatment. Sexual orientation in TM and TW was established by asking what partner (a man, a woman, both or neither) they would prefer or feel attraction to if they were completely free to choose and the body did not interfere. TM and TW underwent endocrinological hormonal assessment to rule out hormonal and sexual development disorders.

To discount the presence of psychiatric disorders and substance abuse within all participants the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) was administered. In addition, cisgender participants were asked for acute or chronic medical conditions, and if they identified themselves as *woman* (CW), *man* (CM) or other non-binary identities.

A total of 29 TM, 17 TW and 41 cisgender individuals (22 CW, 19 CM, and 0 other identities) were finally included in the study. Twenty-four candidates were excluded because: one CM and one TW met criteria of substance dependence; one CM and two CW were on psychoactive medication; two CM, three CW, four TM and two TW did not respond/did not show up on the day of the scan; one CM and three CW had a history of neurological or psychiatric disorders; one CW, one TM and one TW had MRI incompatibilities; and one CM was excluded after resting state preprocessing due to excessive head motion.

Written informed consent was obtained from all participants after full explanation of procedures. The study was approved by the ethics committee of the Hospital Clinic of Barcelona. Resting-state functional and structural MRI data are linked to the Data in Brief article and Mendeley datasets (https://doi.org/10.17632/hjmfrv6vmg.2).

2.2. MRI acquisition and preprocessing

Magnetic resonance images were acquired with a 3T scanner (MAGNETOM Trio, Siemens, Germany), using an 8-channel head coil. The scanning protocol included high-resolution three-dimensional T1-weighted images acquired in the sagittal plane (TR = 2,300 ms, TE = 2.98 ms, TI = 900 ms, 240 slices, FOV = 256 mm; matrix size = 256 × 256; 1 mm isotropic voxel) and a resting-state 10-min-long functional gradient-echo echo-planar imaging sequence (240 T2* weighted images, TR = 2.5 s, TE = 28 ms, flip angle = 80°, slice thickness = 3 mm, FOV = 240 mm). Participants were instructed to keep their eyes closed, not to fall asleep and not to think of anything in particular. Basic functional image preprocessing is described in the Data in Brief article.

For the functional connectivity seed-to-seed and graph theory analyses, we selected 56 ROI from a functional template (Shirer et al., 2012) corresponding to the anterior and posterior SN, dorsal and ventral DMN, sensorimotor network and left and right ECN. ROIs can be found in the Data in Brief files (https://doi.org/10.17632/hjmfrv6vmg.2). We were interested in the connectivity differences within the DMN, the SN, the bilateral ECN and the sensorimotor network. We added the sensorimotor network given previous structural and functional reports in regions of this network (Manzouri et al., 2017; Mueller et al., 2016; Simon et al., 2013; Spizzirri et al., 2018; Zubiaurre-Elorza et al., 2013).

As supplementary analyses, to further support intra and internetwork findings, we performed the same analyses on the 200 ROIs of the functional Craddock's atlas (Craddock et al., 2011). All information related to the Craddock's atlas can be found in the Data in Brief article as for the Shirer et al. (2012) atlas.

2.2.1. Head motion parameters and noise correction

To control for head motion, an exclusion cut-off was established for mean interframe head motion at $\geq$ 0.3 mm translation or 0.3° rotation; and for maximum interframe head motion at $\geq$ 1 mm translation or 1° rotation. As described in the participants section, we excluded one cisman participant due to excessive head movement (maximum rotation: 3.06°).

To remove the effects of head motion and other non-neural sources of signal variation from the functional data, we used an ICA-based strategy for Automatic Removal of Motion Artifacts (ICA-AROMA, Pruim et al., 2015). Data in Brief Table 1 summarizes the groups' means of all motion parameters.

2.3. Statistical analysis

Demographic statistical analyses were conducted using IBM SPSS Statistics 25.0 (2011; Armonk, NY: IBM Corp). We tested for group differences in age and education between groups using Kruskal-Wallis test and pairwise multiple comparisons using Mann-Whitney's *U* test with Bonferroni adjustment.

Three different statistical analyses were performed to address our objectives. We firstly used a whole-brain data-driven methodology via ICA approach with the melodic tool of the FSL software to identify different spatial maps corresponding to different functional connectivity networks. After defining the spatial maps, intergroup statistical differences were assessed within these maps. Second, we assessed the connectivity strength between nodes of distinct networks and finally, we evaluated the global organization of such nodes. For all three approaches,

all possible pairwise group comparisons were performed: CM > CW, CW > CM, CM > TM, TM > CM, CM > TW, TW > CM, CW > TM, TM > CW, TM > TW, TW > TM, that is twelve contrasts (see Fig. 1). For more detailed methods on the functional MRI analyses, please consult the linked Data in Brief article.

2.3.1. ICA spatial maps and dual regression

Melodic (Beckmann and Smith, 2004) from FSL v5.0.10 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) was used to obtain temporal-concatenated spatial maps based on an ICA approach. The SN, the DMN and the bilateral ECN were considered to be the networks of interest. The sensorimotor network could not be identified from any ICA component. Discrepancies in the nomenclature for control networks are frequent in the literature (Gratton et al., 2018). In the present work the network that we defined as bilateral ECN corresponds to the right and left frontoparietal networks described by Smith et al. (2009).

The set of spatial maps from the group-average analysis was used to generate subject-specific versions of the spatial maps, and associated timeseries, using FSL's dual regression (Beckmann et al., 2009; Filippini et al., 2009). We tested for group differences using the FSL permutation-testing tool (5,000 permutations, Winkler et al., 2014) with threshold-free cluster enhancement (TFCE, Smith and Nichols, 2009) including age and education as covariates (age and years of education were confounding variables when comparing TM and CW, and education when comparing TM with CM, see Table 1). Statistical analyses were also conducted without confounding variables (see Data in Brief article). Since we were interested in group connectivity differences within the three selected networks, we applied a binarized mask for each network.

Table 1
Demographics.

	CM (n = 19)	CW (n = 22)	TM (n = 29)	TW (n = 17)	H (Kruskal-Wallis test)	p-value
age	22.0 (7.0)	19.0 (2.0)	22.0 (11.0)	20.0 (3.0)	11.080[a]	0.011
education	13.0 (6.0)	13.0 (2.0)	12.0 (3.0)	14.0 (3.0)	13.522[b]	0.004

Abbreviations: CM, cismen; CW, ciswomen; TM, transmen; TW, transwomen. Data are median and interquartile range.

[a] CW < TM (U = 23.479; P = 0.005) using pairwise Mann-Whitney test adjusted by Bonferroni correction.

[b] CM > TM (U = 23.622; P = 0.006); CW > TM (U = 19.464; P = 0.030) using pairwise Mann-Whitney test adjusted by Bonferroni correction.

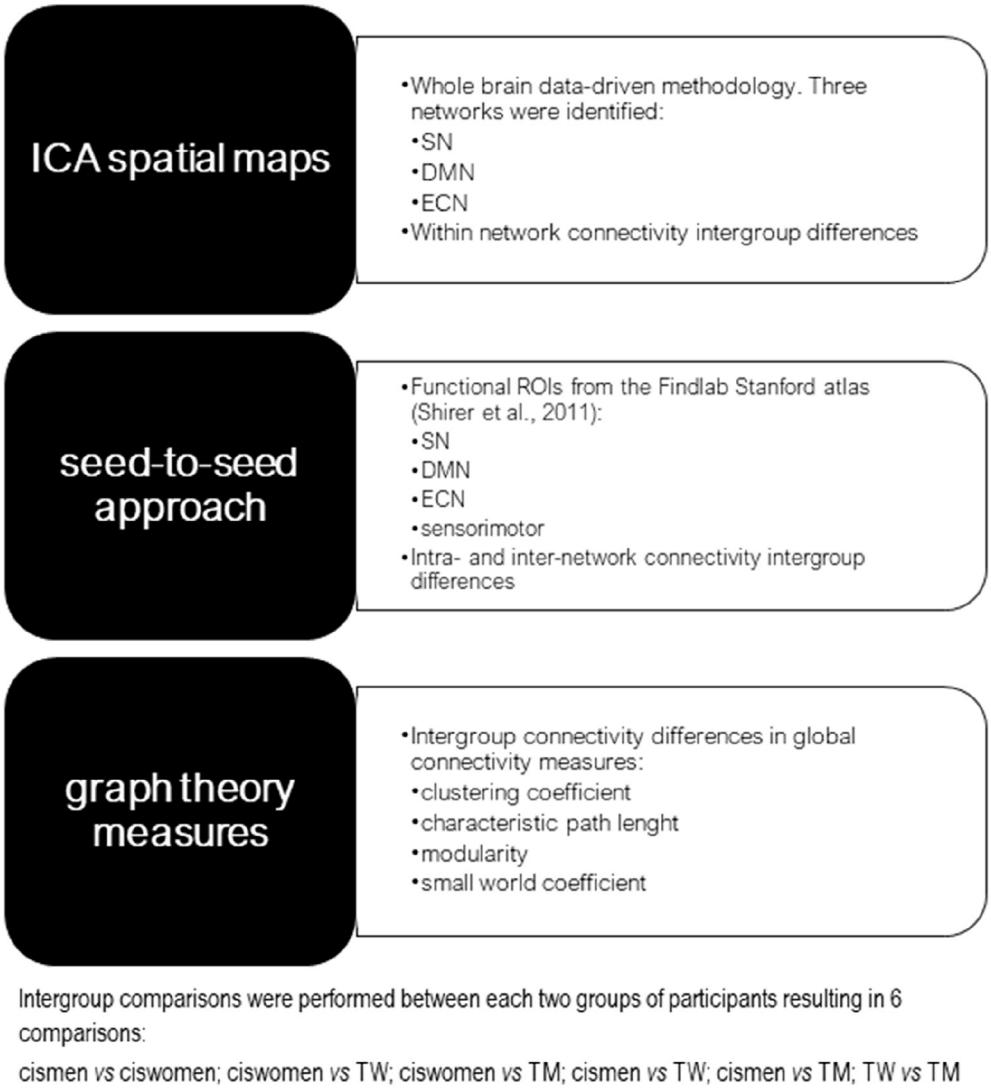

Fig. 1. Summary of the analyses performed in the present study. Abbreviations: DMN, default mode network; ECN, executive control network; ICA, independent component analysis; ROI, regions of interest; SN, salience network; TM, transmen; TW transwomen. The seed-to-seed and the graph theory approaches were additionally tested with the Craddock's atlas (Craddock et al., 2011) as explained in the Data in Brief article.

2.3.2. Intra and internetwork functional connectivity differences

The first eigenvariate of the BOLD signal temporal series was extracted for the 56 ROI from Shirer et al. (2012) with the *fslmeants* command (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Fslutils). The connectivity between two ROI was estimated using Pearson's correlation between their time series. Therefore, a 56 × 56 matrix was obtained for each of the 87 subjects. To test for seed-to-seed intergroup differences in the strength of the edges, we used threshold-free network-based statistics, TFNBS (Baggio et al., 2018). Matlab R2017a (The MathWorks Inc., Natick, MA, USA) was used to perform *t*-test and Montecarlo permutation testing with 1,000 iterations between each of the four groups, including covariates as appropriate (Table 1). All results were also obtained without entering the age and education as covariates (see Data in Brief article). Reported results survived Bonferroni connectome-wise correction for multiple comparisons at $P < 0.05$.

2.3.3. Graph-theory measures

To obtain measures of global connectivity using in-house Matlab script (Baggio et al., 2014), a graph-theory approach was applied using the same matrix (56 × 56 × 87) that we used for the seed-to-seed analyses. Networks were constructed using only positive r values, i.e., setting negative values to 0 (Tian et al., 2007). We used a sparsity threshold to create a set of undirected graphs (existing number of edges in a graph divided by the number of all possible edges) using the r correlation values as edge weights for each pair of seeds for each subject. The range of sparsities was 5–25% with incremental steps of 2.5%. The global graph theory measurements computed were: the *clustering coefficient,* the *normalized global characteristic path length* by 1,000 random networks, *modularity,* and the *small world coefficient.*

Additionally, as supplementary material, we also calculated such measures with an absolute threshold after deleting connections with negative values instead of creating a set of undirected graphs (see Data in Brief).

A general linear model and Monte Carlo permutation testing with 1,000 iterations were applied to test for group differences. Age and education were entered as covariates of no interest in the model as appropriate (age and education were confounding variables when comparing TM and CW, and education when comparing TM with CM, see Table 1). Analyses were also performed without any covariates.

3. Results

TM were older than CW and had fewer years of education than both CM and CW (Table 1). Therefore, all resting-state analyses between these groups were regressed out by these two variables when necessary.

3.1. ICA networks and intergroup differences

The three networks identified through ICA can be seen in Fig. 2. The SN mainly included the bilateral insula, anterior cingulate and paracingulate regions, extending to the pars opercularis, lateral and medial frontal lobe, left supplementary motor area and bilateral supramarginal region. Small clusters in the bilateral cerebellum and bilateral lateral occipital lobe were also part of the independent component. The DMN included bilateral ventral and dorsal medial prefrontal cortex and the posterior cingulate extending to the precuneus and cuneus, supramarginal, supplementary motor area and temporal regions as well as small regions in the cerebellum. The bilateral ECN was divided into two lateralized components that we merged into one network, and mainly included right and left frontoparietal regions. Additionally, the bilateral cerebellum and the bilateral inferior temporal gyrus were also part of this component.

Table 2 summarizes regional group differences within ICA networks that reached $P < 0.05$ after TFCE correction. The CM group had increased connectivity in the left precuneus of the ECN when compared with the CW group (Fig. 3A). A small cluster in the left superior parietal (13 voxels) also reached statistical significance (Table 2).

Similarly, CM also had increased connectivity in the left supramarginal gyrus of the ECN when compared with the TM group (Fig. 3B). Also within the ECN, two small clusters (<40 voxels) in the bilateral frontal pole had increased connectivity in CM with respect to TM (Table 2). Increased connectivity in bilateral supramarginal regions was found within the SN in the CM when comparing them with TM (Fig. 3B).

When comparing the CM group with TW participants, two clusters in the left superior parietal gyrus extending to the supramarginal gyrus, the parietal operculum, and one in the right hemisphere showed increased connectivity within the SN (Table 2, Fig. 3C).

Small clusters (<50 voxels) were found in the left frontal pole within the DMN in TM and TW with respect to CM. There was also a small cluster (10 voxels) of increased connectivity in the right angular gyrus pertaining to the DMN in TW compared with CW.

In summary, CM differed from the other three groups (CW, TM, and TW) in their functional connectivity mainly in superior parietal regions.

3.2. Intra and internetwork functional connectivity analysis

Both intra- and inter-network functional connectivity differences were found between CM and TM, with CM having stronger connectivity than the trans group. A connectogram with the significant connected seeds can be found in Fig. 4A and see Fig. 4B for a topographical representation (see Data in Brief Table 2 for *t*-test stats and P-values of the significant correlations). There were no other seed-to-seed significant differences between any other groups.

The anterior and posterior SN had the greatest number of significant intra- and inter-network differences. Differences in connectivity were mostly found in the frontal lobe with projections to the parietal and temporal lobes and the cerebellum. When comparing intra-network connectivity, two nodes of the anterior salience (both in frontal areas) and another two of the posterior SN (left cerebellum and right middle frontal lobe) had stronger connections. Likewise, there were two more strongly connected nodes from the right ECN located in the frontal lobe in CM than in TM.

Regarding internetwork connectivity, two nodes of the DMN (left paracingulate and right frontal pole) mainly had stronger connections with nodes in the anterior and posterior SN (bilateral prefrontal cortex, insula, left cingulate and right superior parietal). Connectivity differences were also found within the cerebellum when comparing a node in the ventral DMN with two other nodes, one part of the left ECN and the other in the anterior SN. Two nodes of the dorsal DMN located in the lateral occipital and frontal cortex respectively had stronger connections with the left cerebellum, a ROI that was part of the sensorimotor network. Significant differences were found in the left paracingulate (dorsal DMN) with left thalamus (sensorimotor network). While the right ECN had a significant intra-network edge and no internetwork differences, the left ECN (left middle frontal seed) had significant internetwork edges with nodes of the SN located in frontal and parietal regions.

In summary, widespread nodes of the SN, DMN, ECN and sensorimotor networks had decreased intra- and internetwork connections when comparing TM with respect to CM.

Craddock's atlas seed-to-seed analyses (with the 200 × 200 × 87 matrix) can be found in the Data in Brief article.

3.3. Global graph theory measurements

Both CW and TW had significantly less small world coefficients than CM when applying a sparsity threshold to keep 7.5% of the strongest connections. CM also had a higher global clustering coefficient at this same threshold than TW and, when 5% of the strongest connections were kept, the connections also had higher modularity (Fig. 5). No significant differences were found for path length or any other threshold. See Data in Brief Table 3 for means and SD information on the four global measures

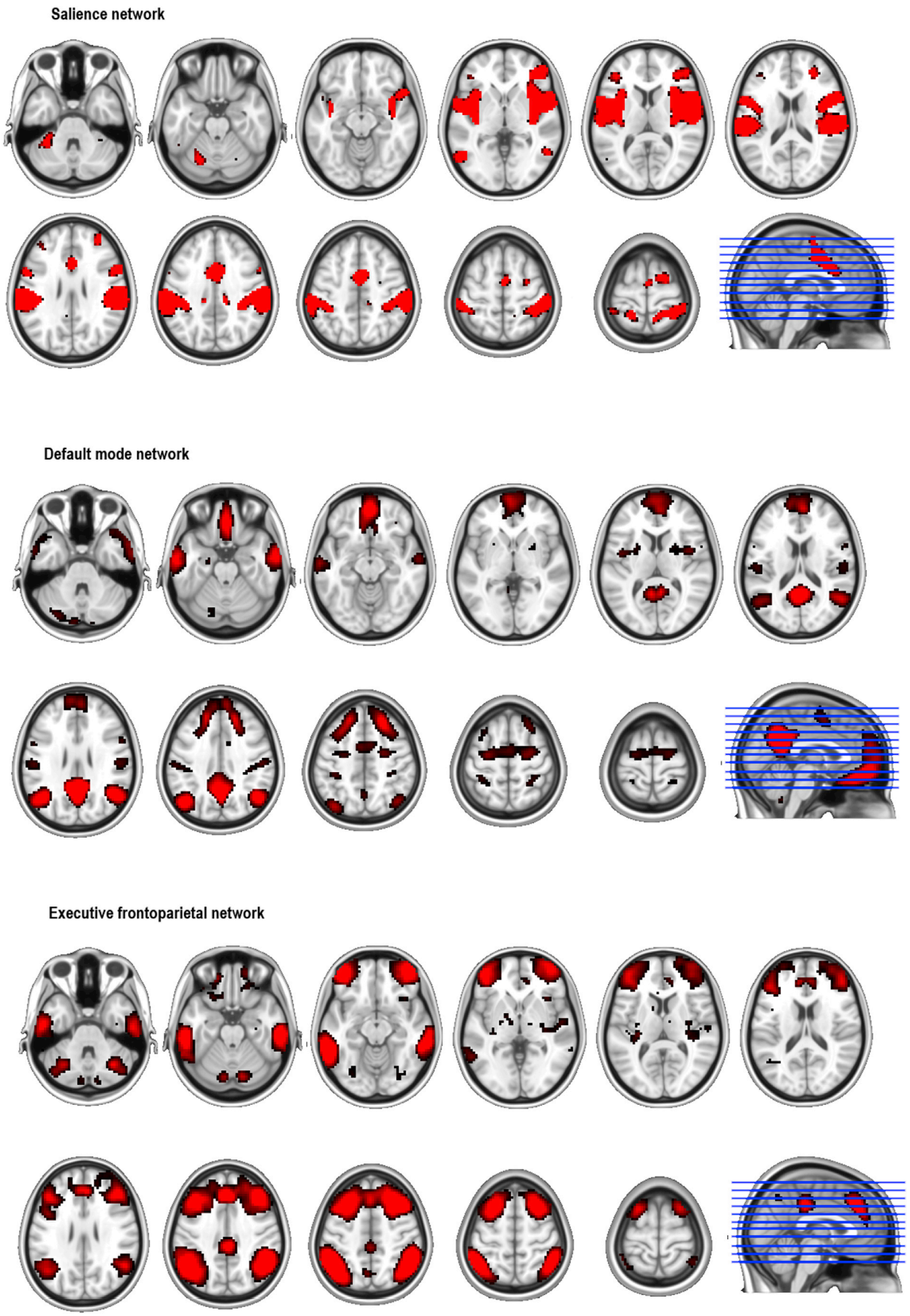

Fig. 2. Functional connectivity networks identified via Independent Component Analysis. Spatial maps were composed from temporal components from the 87 participants included in the study.

Table 2
Significant group differences in ICA networks.

	cluster size (n voxels)	MNI coordinates (x y z)	*t*-test	p-value
salience network				
cismen > transwomen				
L supramarginal Brodmann area 40	136	−51 -39 54	4.040	0.015
L parietal operculum Brodmann area 40	47	−51 -21 15	3.860	0.024
R superior parietal BA primary sensory 1	42	36 -45 66	4.180	0.019
cismen > transmen				
R postcentral Primary somatosensory	168	42 -30 42	4.410	0.004
L supramarginal Brodmann area 40	152	−48 -33 42	5.210	0.002
executive frontoparietal network				
cismen > ciswomen				
L precuneus Brodmann area 39	52	−39 -63 42	3.750	0.029
L superior parietal Brodmann area 40	13	−42 -48 57	3.640	0.041
cismen > transmen				
L supramarginal Brodmann area 40	81	−48 -48 42	3.930	0.022
L frontal pole Brodmann area 10	37	−27 60 15	4.750	0.010
R frontal pole Brodmann area 10	12	24 60 24	4.560	0.030
cismen > transwomen				
R frontal pole Brodmann area 10	23	27 51 18	3.840	0.034
default mode network				
cismen > transwomen				
L frontal pole Brodmann area 10	10	−12 60 27	3.990	0.032
cismen > transmen				
L frontal pole Brodmann area 10	46	−12 60 27	4.510	0.009
L frontal pole Brodmann area 9	5	−15 51 42	3.900	0.041
ciswomen > transmen				
R angular gyrus Brodmann area 39	10	63 -54 27	5.650	0.004

P-values are corrected with threshold free cluster enhancement (TFCE). T-test reported stats of group comparisons that reached statistical significance at P-corrected < 0.05.

at each sparsity threshold.

In summary, CM had higher small worldness, modularity, and clustering coefficient than transwomen and also higher small worldness property than CW.

There were no significant group differences in any graph-theoretical parameter when setting an absolute threshold of 1 (100% connections, see Table 4 in Data in Brief for means and SD information).

Graph-theoretical measures were also obtained with the Craddock's atlas (200 × 200 × 87 matrix). Means, SD and group comparisons information can be found in Table 5 for the relative thresholds and in Table 6 when setting an absolute threshold in the Data in Brief article.

4. Discussion

We investigated the resting state functional connectivity of independent brain component networks, the intra- and inter-network connectivity strengths and global network large-scale characteristics in a homogeneous group of early onset TW and TM. In line with our predictions, there are three main findings: (i) TM, TW, and CW had decreased connectivity in parietal regions compared with CM as we predicted based on the cortical developmental hypothesis; (ii) similarly, TW and CW had less small worldness than CM, showing the existence of gender differences (considering the identification with the gender regardless of the assigned sex at birth) in this global network property; and (iii) TM had weaker connectivity in widespread regions mainly located in the frontal cortex and the majority of their significant connection nodes were part of the SN. Some of the regions that presented reduced functional connectivity in TM in comparison with CM were part of the own's body perception hypothesis.

Our results are in agreement of our previous structural studies in cortical thickness (Zubiaurre-Elorza et al., 2013) and fractional anisotropy (Rametti et al., 2011a) performed in different cohorts, where we proposed the existence of brain phenotypes related to different variants of gender and brain development (for review, see Guillamon et al., 2016; Kreukels and Guillamon, 2016). From the structural perspective, TM, TW, and CW did not differ among each other in cortical thickness, and this is replicated in the current resting state functional connectivity study. This underscores the importance of a developmental point of view addressing the brain in gender variants. Structural and functional MRI data are in disagreement with the popular expectation that transgender people would present an inverted phenotypic brain. Results in relation to the two hypotheses presented in the introduction section will be discussed in the 4.4 section.

4.1. Connectivity phenotype of transmen

4.1.1. ICA results

Regarding ICA, significant differences were seen in the bilateral parietal superior region of the SN and in the left supramarginal region of the ECN when comparing the TM group with the CM group. TM had decreased activation of these regions at rest. The decreased parietal activation in the left ECN is partly like that found in the comparison women group in the same network. Santarnecchi et al. (2012) in a single case study of a TM reported increased and decreased functional connectivity in precuneus and superior parietal regions when compared to both CM and CW. Connectivity differences in the parietal lobe agree with previous structural findings by our group with a different sample in that white matter integrity was increased, reflected by higher FA values in the parietal lobe (Rametti et al., 2011a), and also thickening of this region (Zubiaurre-Elorza et al., 2013). Other authors have also described cortical thinning in the parietal regions (Manzouri and Savic, 2018). Recent works using different methodologies based on functional resting state data have referred to the parietal lobe as a key structure in the own-self body perception processes in TM (Burke et al., 2017; Feusner et al., 2017; Manzouri et al., 2017; Manzouri and Savic, 2018).

In our ICA results, we found a small cluster of higher connectivity in the left frontal as part of the DMN in CM in comparison with TM. Similarly, the Karolinska group reported weaker functional connections within the ventromedial prefrontal cortex (vmPFC) of the DMN comparing TM with respect to cisgender controls (Burke et al., 2017; Feusner et al., 2017). These previous studies were focused on specific ROIs and did not investigate the interaction of the whole brain dynamics.

4.1.2. Seed-to-seed results: brain network interactions

Our seed-to-seed connectivity study adds more complexity to the understanding of the phenotypical differences related to gender rather than the study of isolated regions related to the own body perception. There is a singular connectivity pattern in the TM group that differed from men and that involved decrements in the entire brain but especially weaker connections in the frontal lobe. Group differences in DMN connectivity were found in internetwork functional connections with the ECN, SN and the sensorimotor network. The DMN and the ECN are networks that allow internal and external actions to be respectively processed and they are mutually anticorrelated with the SN, which integrates salience signals in the switch from internally-oriented thoughts to executive external actions (Menon and Uddin, 2010; Uddin, 2015). In addition, although the sensorimotor component could not be identified via ICA, two seeds placed in the thalamus and the cerebellum had weaker

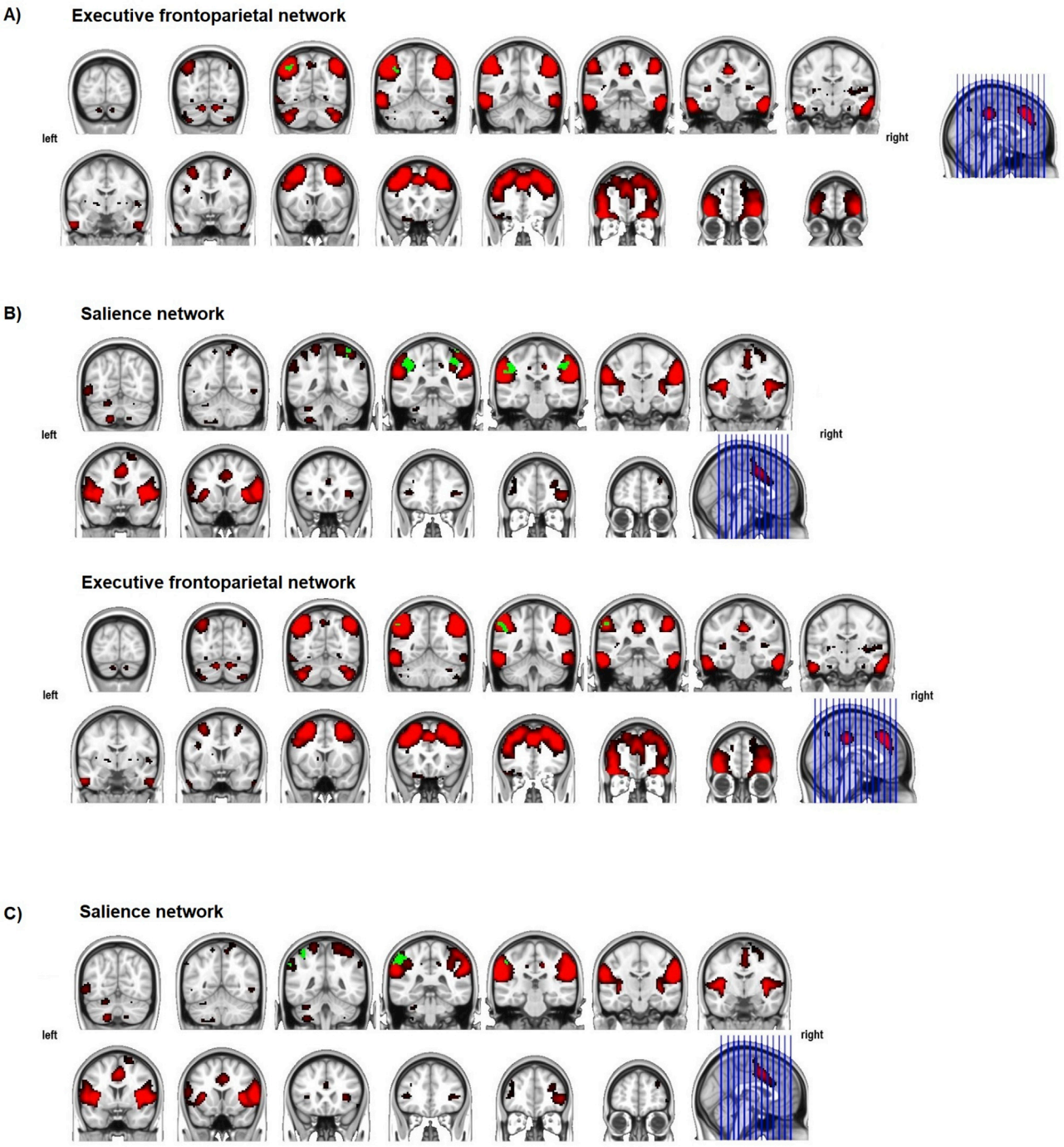

Fig. 3. ICA-functional connectivity maps. A) differences (cismen > ciswomen) in the executive frontoparietal network (ECN); B) differences (cismen > transmen) in the salience network (SN) and the ECN; C) differences (cismen > transwomen) in the SN. In red, the network identified via ICA, including all 87 subjects. In green, clusters with >50 voxels that reached statistical significance at $P < 0.05$ within each spatial ICA network.

connections with nodes in the DMN when comparing TM with CM. This network allows processing of sensory stimuli from the external world and executes direct motor actions. Together, these results suggest that connectivity between internal and external process networks is different in TM than in CM. It would be interesting to investigate if these differences with CM attenuate, disappear or remain after initiating testosterone treatment. One available cross-sectional study pointing in this direction documented that with treatment duration, localized resting-state activity in the cerebellum in transmen approximated that of their gender identity (Mueller et al., 2016).

Connectivity in seeds placed in the insula and the cingulate cortex was weaker in TM than in CM. Previous functional MRI have emphasized the role of the pregenual anterior cingulate cortex with projections to the insula (Burke et al., 2017) as a region that processes perception of one's own body-self (Manzouri et al., 2017).

4.1.3. Graph theory results

We did not find significant differences in global brain connectivity measures (by means of graph theoretical measures) in TM with respect to any of the other sample's groups. In this line, there are two previous

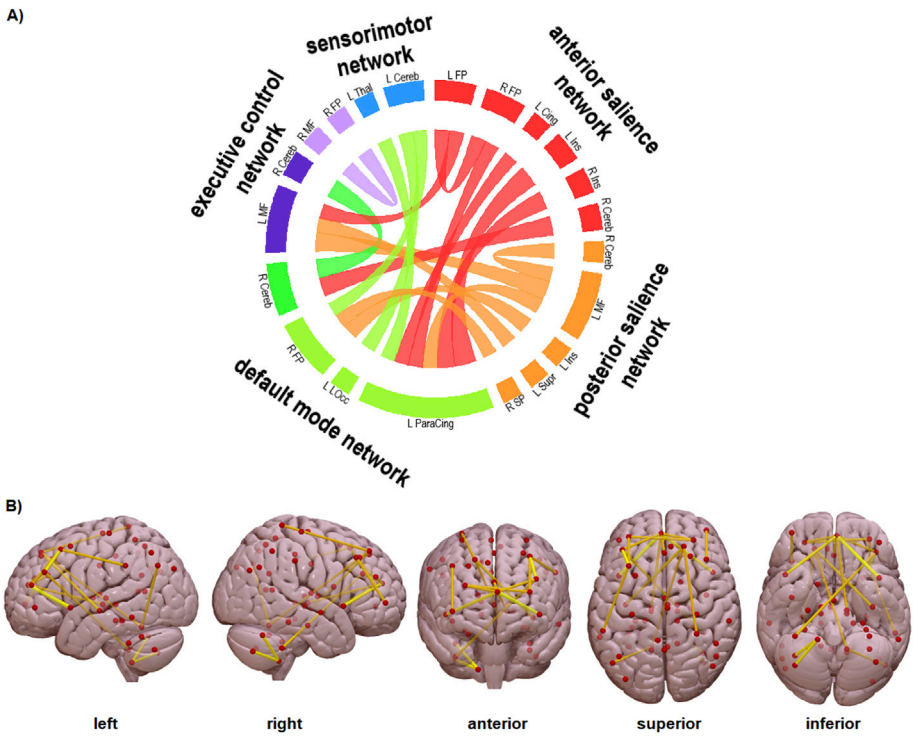

Fig. 4. Functional connectivity differences (cismen > transmen) from the seed-to-seed analysis. A) Connectogram of the significant seeds; each network is represented in a different color. L, left hemisphere; R, right hemisphere; FP, frontal pole; Cing, cingulate; Ins, insula; Cereb, cerebellum; MF, middle frontal; Supr, supramarginal; SP, superior parietal; ParaCing, paracingulate; LOcc, lateral occipital; Thal, thalamus. B) Brain representation. Red dots represent the 56 nodes from the functional template and yellow edges are t tests that reached statistical significance at p-FWE < 0.05 after Montecarlo permutation testing.

studies using graph theory that reported no significant findings in the global network organization in transgender individuals (Hahn et al., 2015; Lin et al., 2014).

In summary, the phenotype of TM is characterized by interaction between networks that include regions related to the own body perception. Additionally, to the classical approaches of connectivity studies, we found that such phenotype is not linked to one specific network but reflects complex relationships between external and internal processing networks.

4.2. Connectivity phenotype of transwomen

4.2.1. ICA results

TW showed decreased activation compared to CM in the left supramarginal cortex in the ICA analysis in SN. These results agree with early fMRI data on task-related activations during the performance of a mental rotation task in which TW had decreased activity in the parietal cortex (Carrillo et al., 2010). Although the differences observed in that work could be due to hormonal treatment, our current data based on resting-state functional networks show that the parietal lobe is a sensitive brain region directly related to the gender identity condition in TW.

4.2.2. Graph theory results

The graph-theory measurements showed that, like CW, the TW group had smaller small world coefficients than CM only when we kept the 7.5% of the strongest connections. They also had a lower clustering coefficient (also at 7.5% of the strongest connections) and modularity (when keeping the 5% of the strongest connections) than did CM. This possibly means that connectivity across the studied functional networks within the CM group follows a higher small-world organization due to a greater functional segregation of the brain. Functional segregation is the ability allowing specialized processing to occur within densely interconnected groups of brain regions and it is measured by the clustering coefficient and modularity properties. On the other hand, no significant differences at any sparsity threshold were found in characteristic path length as a measure for functional integration, i.e. the ability to rapidly combine specialized information from distributed brain regions (Rubinov and Sporns, 2010). Networks that keep a balance between functional integration and segregation are described as having a small-world structure (Sporns and Honey, 2006), meaning that the network has a high clustering coefficient and short path length, that is, global shortcuts across the network that allow fast transfer of information and low energy consumption at the same time (Bullmore and Sporns, 2009). The possible neural underpinnings of an increased/decreased small world network are not clear. Indeed, although a negative correlation is described between increasing age and decreased global connectivity measures in healthy aging studies, such effects are modest and more obvious local changes take place (Gong et al., 2009; Onoda and Yamaguchi, 2013).

In summary, similar to TM, TW showed reduced connectivity in the parietal lobe in comparison with CM. In addition, graph-theory measures are able to differentiate CM from TW and CW in both segregation and integration parameters.

4.3. Connectivity phenotype of cisgender

Increased connectivity was observed in CM in comparison with CW in the left superior parietal gyrus of the executive frontoparietal network from the ICA spatial maps. Two previous studies with large samples of healthy cisgender subjects, have reported functional connectivity differences (Biswal et al., 2010; Ritchie et al., 2018). Interestingly, Biswal et al. (2010) reported sex-related differences in widespread regions of the cerebral cortex, and the superior parietal cortex was a predominantly *male* pattern (male > female) region. In the more recent study (Ritchie et al., 2018), the *male* pattern would present increased connectivity in regions of the sensorimotor network and frontal executive regions (dorsolateral regions). We could not identify a well-defined sensorimotor network although significant differences in the ECN were observed in the superior parietal cortex. On the other hand, the *female* functional pattern is characterized by increased connectivity in the DMN, especially in the posterior parts of the network that are related with the "social brain", and also in medial frontal regions (Ritchie et al., 2018).

In summary, the superior parietal region which is related to the own body perception is showed sex differences.

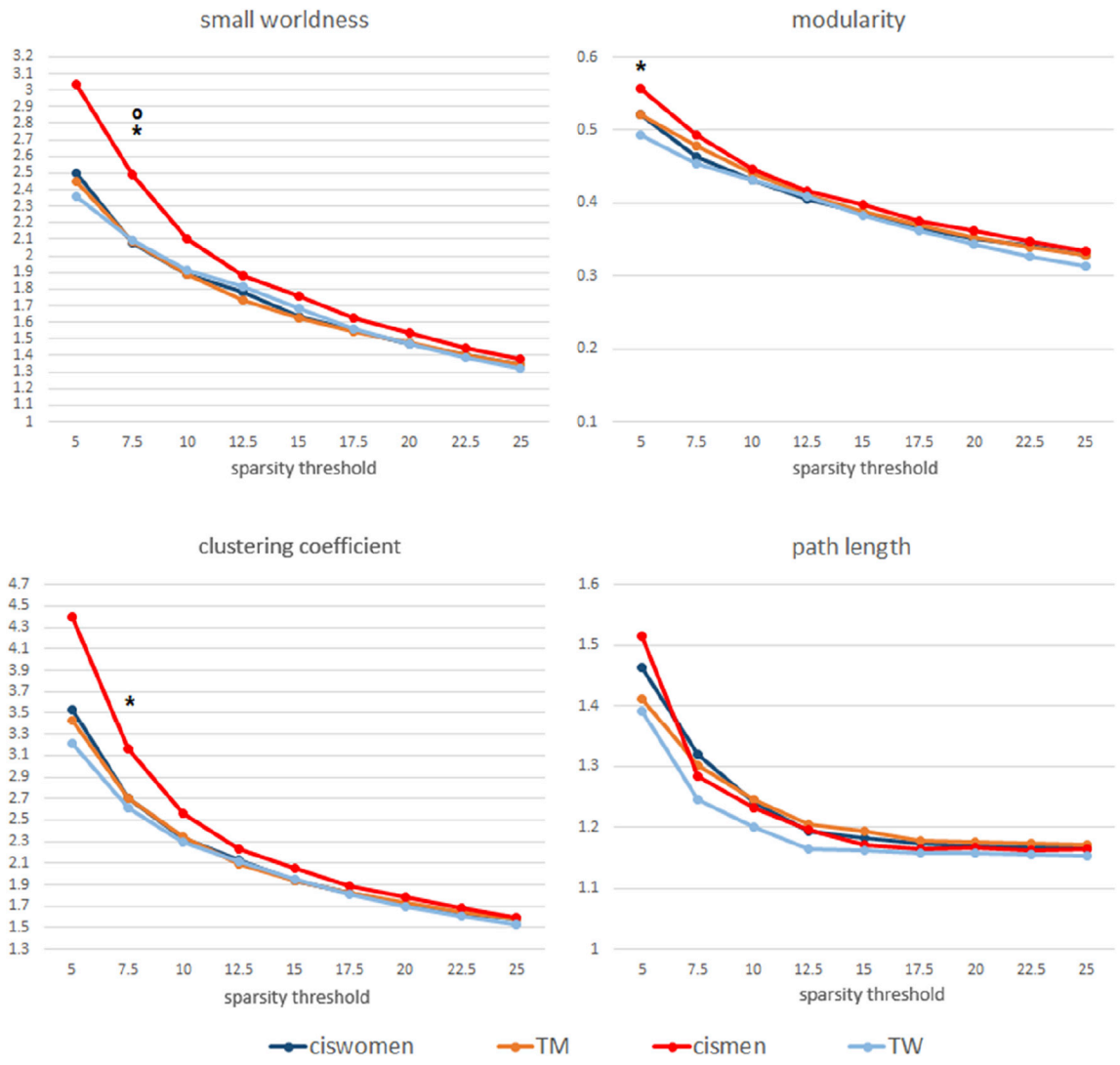

Fig. 5. Graph theory global measurements with different sparsity thresholds. Global clustering coefficient and path length were normalized by 1,000 random iterations. TM, transmen; TW, transwomen.
° cismen > ciswomen; * cismen > transwomen.

4.4. Testing the cortical neurodevelopmental and the own's body perception hypotheses

As mentioned in the introduction, there are two hypotheses that intend to explain brain function in gender variants. The neurodevelopmental hypothesis postulates a slowing in the cortical thinning process in different regions in CW, TW and TM groups with respect to CM (Guillamon et al., 2016); while the own's body perception postulates a fronto-parietal disconnection in transgender people with no involvement of sex differences (Feusner et al., 2017; Manzouri et al., 2017). Our data confirm predictions from both hypotheses and suggest some modifications to the own's body perception hypothesis. On one hand, we found lesser fronto-parietal connectivity strength in TM as predicted by the own's body perception hypothesis; on the other hand, CM differed in connectivity strengths from CW, TW and TM groups which did no differed between them as suggested by the neurodevelopmental hypothesis. Moreover, we found brain networks interactions. Thus, suggesting that these two hypotheses are not contradictory but complementary. This may be due to the different questions they intend to address: the own's body perception hypothesis focus on *how* the gender incongruence is produced whereas the neurodevelopmental one concentrate on *why* it is developed.

The neurodevelopmental cortical hypothesis on gender and its variants has its roots on the effects that sex hormones have on cortical development, and the expression of cortical sex differences. The thickness of the human cortex presents a female > male morphological pattern (Luders et al., 2006; Raznahan et al., 2010; Sowell et al., 2007; Zubiaurre-Elorza et al., 2013). In addition, thickness of CW, TW and TM groups does not differentiate between them, but they do differentiate from CM in several cortical regions (Zubiaurre-Elorza et al., 2013). The possession of an allele conferring more efficient function to the androgen receptor is associated with thinner cortex (Paus et al., 2010; Raznahan et al., 2010). These findings suggested a slowing in the cortical thinning process but in different regions for CW, TW and TM with respect to CM (Guillamon et al., 2016). Moreover, sex hormone receptors polymorphisms have been related to gender variants (Fernández et al., 2018; Henningsson et al., 2005). Analyses of large populations of cisgender and transgender persons found that receptors implicated in the sexual differentiation of the brain have a specific allele combination for α and β estrogen receptors; and the androgen receptor in TW population, whose gender is associated with specific genotypic combination of estrogen and androgen polymorphisms. Further, TM gender is associated with specific polymorphisms of the α and β estrogen receptors. These findings strongly suggest that α and β estrogen receptors play a role in the sexual

differentiation of the brain in humans. Any explanation on how gender is developed ought to offer a common framework for cisgender and all transgender possibilities. The neurodevelopmental hypothesis fulfills this condition since it suggests a gradual mechanism in cortical development for the processes of masculinization, feminization, demasculinization, and defeminization of the brain.

In relation to the networks accounting for gender variants differences, our data confirm the implication of the fronto-parietal network postulated by the own's body perception hypothesis. However, when we compared TM, TW, and cisgender groups one important point emerged. Differences in gender variants were not restricted to a single network but rather to the interaction of several networks. A complex brain function, which controls congruency between the assigned sex at birth and the feeling of being a male or a female, requires at least, a complex relationship between large-scale networks to allow switching between the inner and the outer world. We believe that our results provide the first evidence of such complexities in building up a gendered self.

The main strength of our study is that we used a whole brain functional connectivity approach in searching for interrelational network differences. Initial studies focused on preselected networks or seed-based approaches with *a priori* selected ROI's in untreated TM and TW. Another important aspect is that we studied homogenous groups, with respect to the onset of gender dysphoria, of untreated participants. One limitation of the study would be the relatively small sample size in the TW group, which may have made it difficult to identify a specific pattern of network connectivity. Another limitation was that there were demographic differences between controls and the TM group, similar to those reported in previous studies (Manzouri et al., 2017). However, when data were analyzed with and without age and education as covariates, findings did not differ suggesting little influence of these variables on the present data. Two further limitations regarding the cisgender groups are that we did not control menstrual cycle or contraceptive pill that are associated with altered resting state functional connectivity (Petersen et al., 2014) and we did not ask for their sexual orientation unlike the transgender sample.

5. Conclusions

Our results show the importance of the parietal cortex in both TM and TW concerning the SN and the ECN. Moreover, connectivity in the attentional network is relevant for both TM and TW. With respect to the TM group, interconnectivity between the SN, ECN, and DMN is decreased in comparison with CM. What is more important, these interrelations involve cortical and subcortical nodes over the whole-brain, but with a frontal predominance. Finally, we suggest that the two available hypotheses in the literature on gender identities discussed above are complementary rather than contradictory.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2020.116613.

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